Protection Branch Report of Test No. 11-65

The Level of Microbial Contamination In A Clean Room During A One Year Period

4 December 1964

UNPUBLISHED PRILIMINARY DATA

Approved by:

Donothy M. Porther

Dorothy M. Porther

Decontamination Section

Robert K. Hoffman

ROBERT K. HOFFMAN

Chief, Decontamination Section

Prepared by:

Hard copy (HC) ___

Microfiche (MF) _____.

(CODE)

(ACCESSION NUMBER)

(CODE)

(CODE)

(CODE)

(CATEGORY)

OTS PRICE(S) \$ _____ Charles & Phillips
Charles R. PHILLIPS
Chief, Physical Defense Division

Physical Defense Division Fort Detrick, Frederick, Maryland

Protection Branch Report of Test No. 11-65

The Level of Microbial Contamination In A Clean Room During A One Year Period

15148

The results of a one year study to determine the level of microbial contamination in a clean room are presented in this paper. The purpose methods, and data which were given in an interim report of this study are cited so that this final report will be complete in itself.

A study to determine the level of microbial contamination in a clean room was undertaken in order to establish a basis for deciding whether it is advantageous from a minimal microbial contamination standpoint to assemble a spacecraft in such an area. To increase the probability of obtaining a sterile spacecraft with a given sterilization procedure, precautions must be taken to keep the initial microbial population as low as possible. The level of aerobic microbial contamination that accumulates on surfaces from aerial fallout and also from handling was previously investigated 2 in a laboratory building at this installation, but a more extensive study was needed under clean room conditions. Thus a one year study was undertaken using the clean room facilities of Martin Company, Baltimore, Maryland.

This study was designed only to numerate the viable aerobic and anaerobic microorganisms present. No effort was made to identify the microorganisms. Periodic sampling of the air and aerial fallout for a one or two hour duration was done to give an index of the aerial microbial contamination in the area. The essential part of this study, however, was to determine the number of viable aerobes and anaerobes that accumulate on a stainless steel surface over an extended period. The relative resistance of these microorganisms to heat shocking was also determined. In addition, determinations were made of the microbial

contamination present on stainless steel after handling by gloved clean room personnel because handling may also contribute to the microbial

MATERIALS AND METHODS

Clean Room

contamination.

The clean room (Figure 1) was essentially a complete two room structure (about $50 \times 100 \times 12$ feet) with entry locks, erected in a

corner of a very large factory ($400 \times 1000 \times 49$ feet). At the time these tests were conducted there was relatively little activity in the factory area near the clean room facility. Material was passed into the clean room through the west room or cleaning area (about 50×50 feet) where it was thoroughly cleaned, mainly by ultrasonic vibration, submerged in a bath of trichloroethylene. The trichloroethylene was constantly recirculated through a filter to remove all particles. After the material was cleaned it was passed to the east room or assembly area (about 50×50 feet) where workers put together the various items of equipment under as near dust proof conditions as could be maintained. Finished articles were passed again through the west or cleaning room.

Personnel entered the west or east room through separate dressing areas where they donned clean, lint-free cover-alls, and head and foot coverings. The entire uniform was vacuumed before entering one of the clean rooms. Clean lint-free neoprene gloves were also worn if handling cleaned parts. Each room had its own air circulation system. Although there was a double door passageway between the west and east rooms, traffic through this entry was kept to a minimum. The observed number of people present in the clean room facility during the various test period was always far below the allowable number, 75, based on the ratio of 1.5 persons per 100 square feet. Usually there were less than ten present in the two rooms. The various requirements imposed upon this clean room facility are listed in Tables I, II, and III.

Test Sites

Microbial sampling was conducted in the clean room, both in the west and east rooms, and in the adjacent non-clean factory area, a few feet from the east room of the clean room. The diagram of the floor plan given in Figure 1 shows where the samples were taken.

Microbial Sampling

In this study, the samples* were taken weekly and biweekly for the first two months to observe the trend of the microbial contamination present; the rest of the sampling periods were approximately at five week intervals throughout the year.

* On the day the test started, only the microbial contamination of the air and of the aerial fallout was determined.

Microbial contamination was determined by the following methods:

- 1. Slit samplers $\frac{3}{}$ were used at a flow rate of one cubic foot per minute for an hour duration to collect either the aerobic or the anaerobic microorganisms in the air.
- 2. At the same time interval, agar settling plates (ten to collect aerobes and five to collect anaerobes) were used to determine the number of microorganisms settling from the air.
- 3. A total of 120 sterile 1 x 2 inch stainless steel strips for each test site placed horizontally on sterile stainless steel trays were used to accumulate microorganisms from aerial fallout. At each sampling period listed above, eight strips were assayed to determine the number of viable microorganisms before and after heat shocking. Five of these strips were used to numerate aerobes and the remaining three to numerate anaerobes.
- 4. At each sampling period, four sterile 1 \times 2 inch stainless steel strips were handled by an individual wearing gloves while working in the clean room. Each strip was assayed to determine the number of viable microorganisms before and after heat shocking. Two strips were used to numerate aerobes and two to numerate anaerobes.

Test Procedure

Solid agar plates containing either tryptose* or anaerobic** agar were used in the slit samplers and also for settling plates. These plates were subsequently cultured in the same manner as the pour plates described below.

All stainless steel strip samples were assayed in the same manner. Each strip was placed in a bottle containing 50 milliliters of sterile 0.05 per cent Tween 20 solution. The sample was shaken and then assayed for viable microorganisms by the pour plate method. Thirty milliliters of the sample were plated before the sample was heat shocked at 60° C for 30 minutes, and 15 milliliters were plated after the heat treatment. Each sample was either cultured in tryptose agar under aerobic conditions or in anaerobic agar under anaerobic conditions. All plates for this test were incubated at 37° C for 72 hours before colony counts were made.

Difco Laboratories, Detroit, MichiganBaltimore Biological Laboratory, Baltimore, Maryland

RESULTS AND DISCUSSION

The data collected during the 52 week study are presented in Tables IV through IX. In general, the trend is apparent without special statistical analysis. The number of microorganisms per cubic foot of air was about ten times greater in the factory than in the clean room, and the aerobes seemed to be about five times more prominent than the anaerobes (Table IV). This ratio was also noted in the data showing the rate that airborne microorganisms settle onto a surface (Table V). In various industrial white rooms studied by Michaelson and Vesley 4, the number of microorganisms per cubic foot of air ranged from 7.4 to 0.05. The data reported here also fall within that range.

The data involving the total number of aerobes that accumulated on steel during the year (Tables VI, VII, and VIII) was subjected to statistical analysis to determine (a) if any time trend showed up, and (b) if the evident large difference between factory and clean room attained statistical significance. The anaerobic data was not analyzed since actual numbers were scanty and differences were obvious. There was no significant time trend observed, but the microbial contamination was significantly greater in the factory than in the clean room. More aerobes than anaerobes were present on the steel surface and often times half or more of these microorganisms survived heat shocking. In addition to spore forming bacteria and the occasional molds recovered after heat shocking, some cocci, which were probably protected by extraneous material such as dust, also survived the heat treatment. On the basis of colony formation, a great variety of species were represented.

The data given in Figure 2 shows the mean and the range of five determinations obtained for total aerobic count for each time period and test area. It appears that the microbial population remains fairly constant throughout the year; the statistical results substantiated this observation. The apparent higher contamination level obtained in the factory at 47 and 52 weeks, respectively, appears to be a seasonal phenomenon 2,5 occasioned by a substantial increase in mold population during the late summer months (Table VI).

The microbial population may be increased if the surface is frequently touched by the human hand as occurs during the construction of a spacecraft. Table IX shows that microorganisms were deposited on a previously sterilized stainless steel surface after handling only once with a gloved hand.

The data indicated that, at each test area, the level of microbial contamination obtained on steel after 52 weeks was essentially the same as the level obtained after the first week. This trend was also observed in an earlier study performed in a Fort Detrick laboratory. On the 47th week a new set of sterile steel strips were placed in the factory area and in the clean room areas. These were analyzed on the 52nd week along with those which had been in the test areas for a full year. Table X shows that the aerobic contamination on steel after five weeks exposure to air was comparable to the contamination that accumulated on steel during the entire year.

The results of this study indicate that the microbial contamination is reduced about ten-fold in the Martin Company clean room operating with less than ten per cent of the maximum allowable number of persons for the area. Since a previous study be showed that the microbial contamination in a clean room is greatly increased when occupied to the extent permissible, the microbial contamination could have been greater than indicated here if more personnel were present.

The most striking observation of this study is that the microbial contamination on a stainless steel surface due to aerial fallout rapidly reached a maximum level and remained more or less constant throughout the year.

ACKNOWLEDGEMENT

This study was made possible through the efforts and cooperation of the personnel at the Martin Company.

Thanks are extended to Dr. F.M. Wadley for his critical evaluation and statistical analysis of the data presented in this paper.

References

- 1. Protection Branch Report of Test No. 10-64: The Level of Microbial Contamination in a Clean Room During an Eleven Week Test Period. Physical Defense Division, Fort Detrick, Md. 3 February 1964.
- 2. Protection Branch Report of Test No. 1-64: Microbial Contamination Obtained on Surfaces Exposed to Room Air or Touched by the Human Hand. Physical Defense Division, Fort Detrick, Md. 22 July 1963.
- 3. Wolf, H.W., Skaliy, P., Hall, L.B., Harris, M.M., Decker, H.M., Buchanan, L.M., and Dahlgren, C.M. Public Health Monograph No. 60: Sampling Microbiological Aerosols. U.S. Government Printing Office, 1959.
- 4. Michaelson, G.S. and Vesley, D.: Industrial White Rooms vs Hospital Operating Rooms. Air Eng. 5, 24, sept 1963.
- 5. Kingsley, V.V.: Pharmaceutical Sterile Areas. Part II. Air Eng. 6, 22, July 1964.
- 6. Protection Branch Report of Test No. 1-65: Microbial Contamination In a Clean Room When Occupied by Operating Personnel. Physical Defense Division, Fort Detrick, Md. 12 August 1964.

Table I.

Facility Requirements for The Martin Company Clean Room

- Operations permitted Cleaning, component assembly, component testing, packaging
- 2. Type of flooring covered wall to wall Vinyl
- Smooth, non-flaking walls and ceiling capable of withstanding frequent washings
- 4. Clothing change area
- 5. Double window pass through for parts
- 6. Stainless steel top work benches
- 7. Air conditioning requirements: Temperature control 75 \pm 5F Humidity control 50 \pm 5%
- 8. Degree of positive pressure required:
 Cleaning Area
 O.13" water gauge
 Assembly Area
 O.16" water gauge
- 9. Type of inlet air filtration electrostatic, removing 95% of particles over 5 microns plus a final Cambridge Absolute Filter removing 99.97% of particles greater than 0.3 micron
- 11. Maximum hydrocarbon in air:
 Cleaning area 50 ppm as carbon
 Assembly area 20 ppm as carbon
- * The 2.69 sq in refers to the area of a standard Millipore filter disk.

Table I. (Continued)

- 12. Vaccum outlets from central system
- 13. Gelatin mat shoe cleaner (or equivalent)
- 14. Illumination at bench level Minimum 200 ft. candles
- 15. Air changes required:

 Cleaning area 15/hour, all vapors vented
 Assembly area 14/hour
- 16. Concealed plumbing
- 17. Ocncealed service wiring
- 18. Inter-communication system
- 19. Maximum number of people permitted per 100 ft² of floor space 1.5

Table II.

Area Maintenance and Operational Requirements for Martin Company Clean Room

- Horizontal surfaces shall be vacuumed daily or as required to maintain the specified contamination level. Vertical surfaces shall be vacuumed weekly. Sweeping or dusting shall not be permitted. Use of floor wax is prohibited.
- Walls, floors, ceilings and working surfaces shall be wiped down with synthetic sponges using Dow Fax 9N9, or equivalent, detergent solution at least once each week. Any visible dirt or dust shall be removed immediately.
- 3. Fallout of contamination from the air shall be checked, as necessary, to insure that the room is operating within specified particulate contamination levels.
- 4. A separate room shall be provided for oxidizer and pneumatic work. In addition, the hydrocarbon concentration in the air shall be checked, as deemed necessary by Quality, to insure that the room is operating within process requirements.
- 5. Filters shall be changed when dirty as determined by the pressure drop across the filter, measured with a differential pressure gage, air turn-over less than process requirements, or equivalent method.
- 6. Operating and supervisory personnel shall daily survey the area for unnecessary accumulations of parts, materials, or equipment which sould trap or be possible sources of contamination.
- 7. Eating or use of tobacco in any form shall be prohibited within the area.
- 8. Metal removing tools (e.g., grindstones, files, etc.) shall not be used in the area.
- 9. All unnecessary traffic shall be avoided. Use of inter-com sets is recommended to reduce personnel traffic within the area.
- 10. The particulate contamination level of each clean room shall be posted at the entrance to the area.

Table III.

Personnel Clothing and Hand Tool Requirements For Martin Company Clean Room

- 1. All personnel certified to enter clean room shall don clean, lintfree cover-alls, and head covering in the clothing change area and vacuum the entire uniform, including shoes, prior to entering the clean area.
- 2. Personnel leaving the clean room for any reason must remove all protective clothing in the dressing room. Upon returning, proper protective clothing shall be donned and cleaned, as in item 1, above, before re-entry into the clean room.
- 3. When handling cleaned parts, used in oxidizer systems, clean lintfree neoprene gloves shall be worn.
- 4. Hand tools shall be cleaned and packaged in accordance with Process p-50051. Tools used to work hydraulic or fuel components shall not be used for pneumatic or oxidizer components unless they are recleaned. Tools containing wood or with serrated or knurled jaws are prohibited except in semi-conductor research areas.
- 5. No visitors shall enter the area.
- 6. When necessary for special work purposes, an uncertified person may enter the area provided that he obtains permission from the clean room supervisor who shall instruct him in clean room procedures and escort him at all times to ensure that the procedures are followed.

Table IV. Number of Microorganisms Per Cubic Foot of Air in Test Areas

	Anaerobes	Total	0.2	0.07	0.05	0	0.1	0.08	0.2	0.08	0.03	0.02	0.02	0	0.02	0.03	0.07	0.2
Assembly Area		Total	0.8	0.2	0.5	0.07	4.0	4.0	4.0	4.0	4.0	9.0	4.0	0.2	0.8	1.2	0.2	0.7
Assem	Aerobes	Mold	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3
	Ă	Bacteria	0.8	0.2	0.5	0.07	4.0	4.0	4.0	4.0	4.0	9.0	4.0	0.2	8.0	1.2	0.2	0.4
t of Air	Anaerobes	Total	0.2	0.03	0	0.05	0.1	0.02	0.08	0.02	0	0.03	0.02	0.02	0	0.02	0.05	0.3
Microorganisms/Cubic Foot of Factory Area		Total	8.0	4.0	0.7	0.2	4.0	0.08	0.7	0.7	4.0	0.2	0.2	0.2	0.2	4.0	0.2	9.0
	Aerobes	Mold	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1
	Aer	Bacteria	8.0	4.0	0.7	0.2	4.0	0.08	0.7	7.0	4.0	0.2	0.2	0.2	0.2	7.0	0.2	0.5
	Anaerobes	Total	0.3	1.4	1.7	2.4	0.3	1.2	1.0	0.5	0.08		0.3	0.03	0.3	0.08	0.2	
		Totaî	3.6	3.4	4.9	7.3	2.9	3.3	3.9	3.2	2.3	2.4	3.8	1.2	4.0	3.0	9.1	3.2
	Aerobes	Moid	0.8	0.2	0.3	0.1	0.1	0.1	0.2	0	0.1	0.1	0.2	0	0.3	0.2	7.0	1.0
	Ae	Bacteria	2.8	3.2	7.6	7.2	2.8	3.2	3.7	3.2	2.2	2.3	3.6	1.2	3.7	2.8	1.2	2.2
		Date	9/24/63	10/1/63	10/8/63	10/15/63	10/22/63	11/ 5/63	11/19/63	12/10/63	1/21/64	2/25/64	3/31/64	4/58/64	6/2/64	7/14/64	49/81/8	9/22/64

Note: Each entry represents one determination.

Number of Microorganisms Settling Per Square Foot Per Hour in Test Areas Table V.

Object Anaer Object		Factory	/ Area		Microorg	ganisms/ Cleaning	Square Area	Microorganisms/Square Foot/Hour Cleaning Area		Assembly	bly Area	
Total Total Bacteria Mold Total Bacteria Mold Total 178 12 64 2 66 34 46 0 46 322 81 18 2 20 4 11 0 46 271 102 4 0 4 0 9 0 9 376 80 15 0 4 0 9 0 9 157 18 37 12 20 0 5 0 5 157 18 37 12 20 5 0 5 0 5 192 24 19 0 15 4 18 0 18 192 20 16 16 16 2 16 16 16 351 22 23 16 16 16 17 16 17 192 42 <th>Aerobes</th> <th>bes</th> <th></th> <th>Anaerobes</th> <th>A</th> <th>erobes</th> <th></th> <th>Anaerobes</th> <th>Ae</th> <th>robes</th> <th></th> <th>Anaerobes</th>	Aerobes	bes		Anaerobes	A	erobes		Anaerobes	Ae	robes		Anaerobes
178 12 64 2 66 34 46 0 46 2 322 81 18 2 20 4 11 0 11 0 11 0 11 0 11 0 11 0 11 0 11 0 11 0 11 0 11 0 11 0 11 0 11 0 11 0	Bacteria Mo	old	Total	Total	·	Mold	Total	Total		Mold	Total	Total
322 81 18 2 20 4 11 0 11 271 102 4 0 4 0 9 0 9 376 80 15 0 15 4 5 0 5 157 18 37 12 20 5 0 5 250 33 5 0 5 0 15 1 16 304 24 19 0 19 4 18 0 20 192 20 18 0 18 0 18 0 18 192 20 16 16 3 15 0 15 0 15 236 42 16 16 2 71 0 15 1 167 18 0 16 2 31 0 15 1 167 40 16		17	178	12	479	7	99	34	9†7	0	94	24
271 102 4 0 4 0 4 0 9 0 9 376 80 15 0 15 4 5 0 5 250 18 0 37 12 20 0 20 304 24 19 0 19 4 18 0 18 192 20 18 0 18 0 18 0 18 192 38 16 0 16 19 0 18 0 18 351 22 18 0 16 10 0 10	312	0	322	81	18	2	20	4	-	0	=	2
376 80 15 0 15 4 5 0 5 157 18 37 12 20 0 20 250 33 5 0 5 0 15 1 16 304 24 19 0 19 4 18 0 18 0 18 0 18 0 10 <td< td=""><td>256</td><td>15</td><td>271</td><td>102</td><td>4</td><td>0</td><td>7</td><td>0</td><td>6</td><td>0</td><td>σ</td><td>7</td></td<>	256	15	271	102	4	0	7	0	6	0	σ	7
157 18 37 0 37 12 20 0 20 250 33 5 0 5 0 15 1 16 304 24 19 0 19 4 18 0 18 192 20 18 0 10 0 10 10 192 38 16 0 15 0 15 0 15 236 42 15 0 20 15 0 15 0 15 0 15 0 15 0 15 0 15 0 15 0 15 0 15 0 15 0 15 0 15 0 15 0 15 0 15 0 17 0 17 0 17 0 17 0 17 0 17 0 17 0 17 0 17 0<	367	6	376	80	15	0	15	4	5	0	٧.	4
250 33 5 0 5 0 15 1 16 304 24 19 0 19 4 18 0 18 192 20 18 0 18 0 18 0 18 192 38 16 0 16 0 15 0 15 15 236 42 15 0 15 0 15 0 15 0 15 0 15 15 1	153	7	157	18	37	0	37	12	20	0	20	9
304 24 19 0 19 4 18 0 18 192 20 18 0 16 0 16 0 10 0 10 192 38 16 0 16 3 15 0 15 15 0 15 0 15 0 15 0 15 0 15 0 11 11 11 11 11 11 11 11 11 11 11 11 11 11 1	242	∞	250	33	2	0	72	0	15	-	91	0
192 20 18 0 18 0 16 0 10 10 10 10 10 10 10 10 10 10 10 15 0 15 0 15 0 15 0 15 0 11 11 11 11 11 <td>300</td> <td>4</td> <td>304</td> <td>24</td> <td>61</td> <td>0</td> <td>19</td> <td>4</td> <td>18</td> <td>0</td> <td>18</td> <td>47</td>	300	4	304	24	61	0	19	4	18	0	18	47
192 38 16 0 16 3 15 0 15 351 22 29 0 29 2 71 0 71 236 42 15 3 6 0 71 79 8 16 0 16 2 31 0 6 167 18 0 16 0 17 0 17 210 40 38 0 38 49 0 49 106 2 10 0 10 0 17 0 49 204 46 50 4 54 6 35 2 37	190	7	192	20	81	0	18	0	10	0	10	m
351 22 29 0 29 2 71 0 71 236 42 15 3 6 0 6 79 8 16 0 16 2 31 0 31 167 18 8 0 8 17 0 17 210 40 38 0 38 49 0 49 1 106 2 10 0 10 0 19 4 23 204 46 50 4 54 6 35 2 37	192	0	192	38	91	0	16	m	15	0	15	10
236 42 15 0 15 3 6 0 6 79 8 16 2 31 0 31 167 18 8 6 17 0 17 210 40 38 8 49 0 49 1 106 2 10 0 10 0 19 4 23 204 46 50 4 54 6 35 2 37	347	4	351	22	29	0	59	7	7.1	0	11	2
79 8 16 0 16 2 31 0 31 167 18 8 6 17 0 17 210 40 38 6 17 0 17 106 2 10 0 38 8 49 0 49 1 204 46 50 4 54 6 35 2 37		7	236	42	15	0	15	m	9	0	9	2
167 18 8 0 8 6 17 0 17 210 40 38 0 38 49 0 49 1 106 2 10 0 10 0 19 4 23 204 46 50 4 54 6 35 2 37	79	0	79	∞	91	0	91	2	31	0	31	2
210 40 38 0 38 49 0 49 1 106 2 10 0 10 0 4 23 204 46 50 4 54 6 35 2 37	164	m	167	18	∞	0	∞	9	17	0	17	2
106 2 10 0 10 4 23 204 46 50 4 54 6 35 2 37	194	16	210	04	38	0	38	∞	64	0	64	12
204 46 50 4 54 6 35 2 37	77	29	106	2	10	0	10	0	19	4	23	2
	159	45	204	947	50	4	54	9	35	2	37	9

Each entry represents one determination . Note:

Table VI. Microbial Contamination Obtained on Stainless Steel Exposed to Air in the Factory Area During a 52 Week Period

Agrobes Anaerobes Anaerobes Anaerobes 7,900 300 8,200 40 1,200 # 1,200 5,200 400 5,600 80 1,900 100 2,200 4,000 700 4,700 120 2,400 100 2,500 5,200 400 5,800 120 2,800 100 2,900 5,200 400 5,800 120 2,800 100 2,900 6,500 500 7,000 520 4,000 2,900 4,200 8,500 400 6,300 280 8,300 600 8,900 5,500 400 6,200 480 5,500 4,400 100 4,500 5,500 7,00 6,200 480 5,500 4,400 100 4,500 5,300 400 5,700 200 3,000 100 3,100 5,300 400 6,400 1,000 4,400 100			No Treatment	tment	Microorga	Microorganisms/Square Foot	Treatment:	ent: Heat	
Bacteria Mold Total Total Total Hold Total Total <t< th=""><th>Date & Weeks</th><th></th><th>Aerobes</th><th></th><th>Anaerobes</th><th></th><th>Aerobes</th><th>ł</th><th>Anaerobes</th></t<>	Date & Weeks		Aerobes		Anaerobes		Aerobes	ł	Anaerobes
(1) 7,900 300 8,200 40 1,200 # 1,200 (2) 5,200 400 5,600 80 1,900 100 2,200 (3) 4,000 700 4,700 120 2,400 100 2,500 (4) 3,500 1,000 4,500 160 2,300 300 2,600 2,500 (6) 5,200 6,500 5,800 120 2,300 4,000 2,500 2,500 (8) 6,500 5,000 2,000 2,20 4,000 2,300 4,200 3,300 (11) 5,300 400 6,200 280 8,300 600 8,900 4,500 (21) 5,300 400 1,000 3,100 4,400 100 4,500 8 (31) 5,300 400 6,400 1,000 2,000 3,100 3,100 3,300 (42) 7,000 12,000 12,000 12,000 1	of Exposure	Bacteria	Mold	Total	Total	Bacteria	Mold	Total	Total
(2) 5,200 400 5,600 80 1,900 100 2,200 (3) 4,000 700 4,700 120 2,400 100 2,500 (4) 3,500 1,000 4,500 160 2,300 300 2,600 2,500 (6) 5,200 600 5,800 120 2,800 100 2,900 2,600 3,000 2,900 3,500 3,500 4,200 3,500 3,500 4,200 3,500 4,200 3,500 4,200 3,500 4,200 3,500 4,200 3,500 4,200 3,500 4,200 3,100 4,200 3,100 4,200 3,100 4,200 3,100 3,100 3,100 4,200 3,100 3,100 4,200 4,200 3,100 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200 4,200	10/ 1/63 (1		300	8,200	04	1,200	#	1,200	#
(3) 4,000 700 4,700 120 2,400 100 2,500 (4) 3,500 1,000 4,500 160 2,300 300 2,500 (8) 5,200 5,800 120 2,800 100 2,900 (11) 5,900 4,00 520 4,000 200 4,200 (11) 8,500 4,00 280 8,300 8,300 8,300 (11) 8,500 7,00 280 8,300 8,300 8,500 (12) 8,500 4,80 5,500 4,400 1,00 4,500 (22) 7,200 8,00 4,400 1,00 4,500 (31) 5,300 4,00 1,000 3,00 1,00 4,500 (42) 5,800 6,00 1,000 1,00 1,00 1,00 1,00 1,00 1,00 1,00 1,00 1,00 1,00 1,00 1,00 1,00 1,00 1,00 1,00	10/8/63 (2		700	5,600	80	1,900	100	2,200	80
(4) 3,500 1,000 4,500 160 2,300 300 2,600 (8) 5,200 600 5,800 120 2,800 100 2,900 (11) 5,900 400 6,300 200 4,000 2,000 4,200 (11) 5,900 400 6,300 280 8,300 600 8,300 (17) 8,500 700 6,200 480 5,500 4,400 100 4,500 (27) 7,200 300 7,500 200 4,400 100 4,500 (31) 5,300 400 5,700 200 3,000 100 4,500 (42) 5,800 600 6,400 1,000 3,300 4,900 4,900 (42) 7,000 12,000 18,000 120 4,700 2,100 (52) 7,000 12,000 12,000 12,000 12,000 12,000			700	4,700	120	2,400	100	2,500	#
(8) 5,200 b 600 5,800 120 2,800 100 2,900 (8) 6,500 b 500 7,000 520 4,000 200 4,200 (11) 5,900 b 400 6,300 200 2,100 2,300 4,200 (17) 8,500 b 500 9,000 b 480 8,300 b 8,900 8,900 (22) 5,500 b 7,500 b 480 b 6,900 b 4,900 b 4,900 b 4,500 b 4,500 b (31) 5,300 b 400 b 5,700 b 200 b 4,900 b			1,000	4,500	160	2,300	300	2,600	240
6,500 500 7,000 520 4,000 200 4,200 5,900 4,00 200 2,100 200 2,300 8,500 5,00 280 8,300 6,900 8,900 5,500 700 4,80 6,500 8,900 8,900 7,200 300 7,500 200 4,400 100 4,500 5,300 400 5,700 200 3,000 100 3,100 5,800 600 6,400 1,000 4,900 4,900 4,900 3,500 4,000 16,000 80 4,700 4,700 7,000 12,000 12,000 120 6,900 7,100	$\overline{}$		009	5,800	120	2,800	100	2,900	#
5,900 400 6,300 200 2,100 2,300 8,500 500 9,000 280 8,300 600 8,900 3 5,500 7,200 480 5,500 4,400 100 4,500 4 5,300 400 5,700 200 3,000 100 4,500 8 5,800 600 6,400 1,000 4,900 4,900 8 8 8,300 8 7,000 12,000 12,000 12,000 12,000 6,900 7,100 7,100 7,100	_		200	7,000	520	4,000	200	4,200	320
8,500 5000 280 8,300 600 8,900 5,500 700 6,200 480 5,500 # 5,500 7,200 300 7,500 200 4,400 100 4,500 5,300 400 5,700 200 3,000 100 3,100 5,800 600 6,400 1,000 4,900 # 4,900 3,500 12,000 16,000 80 4,700 7,000 7,100	12/10/63 (11		400	6,300	200	2,100	200	2,300	80
5,500 700 6,200 480 5,500 #,400 100 4,500 7,200 300 7,500 200 3,000 100 4,500 5,300 400 5,700 200 3,100 3,100 5,800 600 6,400 1,000 4,900 # 4,900 3,500 4 3,500 4,700 4,700 4,700 7,000 12,000 120 6,900 7,100	1/21/64 (17		200	9,000	280	8,300	009	8,900	320
7,200 300 7,500 200 4,400 100 4,500 5,300 400 5,700 200 3,000 100 3,100 5,800 600 6,400 1,000 4,900 # 4,900 8 3,500 # 3,300 # 3,300 7,000 12,000 16,000 120 6,900 7,100	2/25/64 (22		700	6,200	084	5,500	#	5,500	004
5,300 400 5,700 200 3,000 100 3,100 5,800 600 6,400 1,000 # 4,900 # 4,900 3,500 # 3,300 # 3,300 7,000 9,000 16,000 80 #,700 7,100 7,000 12,000 19,000 120 7,100	3/31/64 (27		300	7,500	200	4,400	100	4,500	80
5,800 600 6,400 1,000 # 4,900 # 4,900 3,500 # 3,500 120 # 3,300 # 3,300 7,000 9,000 16,000 80 # 4,700 # 4,700 7,000 12,000 19,000 120 6,900 200 7,100	4/28/64 (31		004	5,700	200	3,000	100	3,100	#
3,500 # 3,500 120 3,300 7,000 9,000 16,000 80 4,700 # 4,700 7,000 12,000 120 6,900 200 7,100	6/ 2/64 (36		009	6,400	1,000	4,900	#	4,900	800
7,000 9,000 16,000 80 4,700 # 4,700 7,000 12,000 19,000 120 6,900 200 7,100 8	7/14/64 (42		#	3,500	120	3,300	#	3,300	80
7,000 12,000 19,000 120 6,900 200 7,100	8/18/64 (47		9,000	16,000	80	4,700	#	4,700	#
	9/22/64 (52)		12,000	19,000	120	6,900	200	7,100	80

surface, as many as 19 microorganisms could have been present and not detected by the assay method. Note: Each entry for the aerobes is based on 5 determinations (graphically presented in Figure 2) and on No colonies were observed from the assay of 6 or 10 sq in. of surface; but on the basis of a sq ft of 3 for the anaerobes. \ * *

~

Table VII. Microbial Contamination Obtained on Stainless Steel Exposed to Air in the. Cleaning Area During a 52 Week Period

				Microorgan	Microorganisms/Square Foot			
		No Treat	tment			Treatment:	ent: Heat*	
Date & Weeks		Aerobes		Anaerobes		Aerobes	1	Anaerobes
of Exposure	Bacteria	Mold	Total	Total	Bacteria	Mold	Total	Total
10/ 1/63 (1)	1,700	#	1,700	#	90	#	50	#
10/ 8/63 (2)	580	120	700	04	96	8	180	#
10/15/63 (3)	450	20	740	04	#	*	#	#
10/22/63 (4)	340	#	340	#	96	#	06	#
(9) 89/5 /11	360	#	360	#	#	#	#	#
11/19/63 (8)	930	#	630	120	50	#	50	#
12/10/63 (11)	1,100	#	1,100	1,200	96	#	96	#
1/21/64 (17)	270	110	380	#	50	#	50	#
2/25/64 (22)	340	20	360	#	50	#	50	#
3/31/64 (27)	380	20	400	#	140	#	071	#
4/28/64 (31)	450	#	450	#	50	#	50	#
6/ 2/64 (36)	240	#	240	#	06	#	96ء	#
7/14/64 (42)	270	20	290	#	190	#	190	160
(/4) 49/81/8	430	310	740	#	240	#	240	#
	450	09	510	#	50	#	50	#
* Exposed to 6	60 C for 30 minutes.	nutes.	· ·					

surface, as many as 19 microorganisms could have been present and not detected by the assay method. e: Each entry for the aerobes is based on 5 determinations (graphically presented in Figure 2) and on 3 No colonies were observed from the assay of 6 or 10 sq in. of surface; but on the basis of a sq ft of for the anaerobes. Note: 9 * #

Microbial Contamination Obtained on Stainless Steel Exposed to Air in the Assembly Area During a 52 Week Period Table VIII.

		No Treatment	ment			Treatment:	ent: Heat*	*
Date & Weeks		Aerobes		Anaerobes		Aerobes		Anaerobes
of Exposure	Bacteria	Mold	Total	Total	Bacteria	Mold	Total	Total
10/ 1/63 (1)	50	#	50	0†	380	#	380	#
10/ 8/63 (2)	450	#	450	07	340	#	340	#
10/15/63 (3)	90	#	96	0†	06	#	06	80
10/22/63 (4)	70	#	70	04	06	#	96	#
11/ 5/63 (6)	190	*	190	0†	190	#	190	#
11/19/63 (8)	120	20	140	#	#	#	#	#
12/10/63 (11)	170	#	170	#	#	#	#	#
1/21/64 (17)	630	#	630	#	240	#	240	#
2/25/64 (22)	530	#	530	#	140	#	140	#
3/31/64 (27)	240	#	240	#	50	#	50	#
4/28/64 (31)	290	#	290	#	190	#	190	#
(96) 49/2 /9	270	#	270	120	240	#	240	#
7/14/64 (42)	170	50	220	0†	#	#	#	80
(24) 49/81/8	260	50	310		190	#	190	ı
9/22/64 (52)	140	140 120	260	#	90	#	90	#

* Exposed to 60 C for 30 minutes. - Not assayed.

No colonies were observed from the the assay of 6 or 10 sq in. of surface; but on the basis of a sq ft of surface, as many as 19 microorganisms could have been present and not detected by the assay method.

Note: Each entry for the aerobes is based on 5 determinations (graphically presented in Figure 2) and on 3 for the anaerobes.

<u>ν</u>

Average Number of Microorganisms on Stainless Steel After Handling With A Gloved Hand by Personnel in Clean Room Table IX.

Anaerobes Aerobes 0 # 120 # 120 0 # 240 # 240 0 # # # # 0 # # # # 0 # # # # 0 # # # # 0 # # # # 0 # # # # 0 # # # # 0 # # # # # 0 # # # # # 0 # # # # # 0 # # # # # 0 # # # # # 0 # # # # # 0 # # # # #			No Treatment	tment	Micro	Microorganisms/Square Foot		Treatment:	Heat	
Total Total Bacteria Mold Total Total Total Total Bacteria Mold Total Tota			Aerobes		Anaerobes		Ae	robes		Anaerobes
180 # 120 # 120 180 # 240 # 240 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # # 180 # # # #	Date	Bacteria	Mold	Total	Total	Bacter		lold	Total	Total
180 # 240 # 240 180 # # # # 180 # # # # 60 # 120 # # 240 # # # # 60 # # # # 660 # 120 # # 660 # 120 # # 660 # 240 # 240 660 # 240 # # 660 # # # # 660 # # # # 660 # # # # 660 # # # # 660 # # # # 660 # # # # 660 # # # # 660 # # # # 660 # # # # <t< td=""><td>10/ 1/63</td><td>180</td><td>#</td><td>180</td><td>*</td><td>120</td><td></td><td>#</td><td>120</td><td>#</td></t<>	10/ 1/63	180	#	180	*	120		#	120	#
180 #	10/ 8/63	09	#	9	*	240		#	240	#
180 # 120 120 60 # # # # 240 # # # # 240 # # # # 60 # # # # 60 # # # # 60 # 120 # # 60 # 240 # # 60 # # # # 60 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # # 120 # # # # # 120 # # # # #<	10/15/63	120	09	180	*	#		#	#	120
60 # 120 # 120 # # # # # 240 # # # # 540 # # # # 60 # # # # 650 # # 240 # 60 # # # # 60 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 120 # # # # 1	10/22/63	180	#	180	*	#		120	120	#
4 #	11/ 5/63	09	#	9	#	120		#	120	#
240 #	11/19/63	#	#	#	*	#		#	#	#
240 # 480 360 840 60 # # # # 660 # # # # 60 # # # # 60 # 240 # # 60 # # # # 60 # # # # 120 # # # #	12/10/63	#	#	#	*	#		#	#	#
60 #	1/21/64	240	#	240	#	1480		360	840	#
60 # 120 # 120 660 # # # # 60 # # # # 60 # # # # 120 # # # #	2/25/64	09	#	09	#	#		#	#	#
660 # # # # # 60 # # # # 60 # # # # 120 # # # #	3/31/64	09	*	09	#	120		#	120	#
# # 240 # 240 60 # # # # 60 # # # # 120 # # # #	4/58/64	099	#	099	#	#		#	#	#
60 # # # # # 120 # # #	t9/2/9	#	#	#	#	240		#	240	#
60 # # # # # 120 # # # # # # # # # # # # # # # # # # #	49/41//	09	#	09	#	#		#	#	#
120 # # #	8/18/64	09	#	09	#	#		#	#	#
	9/22/64	120	#	120	#	#		#	#	#

No colonies were observed from the assay of 4 sq in. of surface; but on the basis of a sq ft of surface, as many as 59 microorganisms could have been present and not detected by the assay method. #

Each entry is based on 2 determinations. Note:

Table X.

Aerobic Contamination Obtained on Stainless Steel After Five Weeks* Exposure to Air in the Factory and Cleaning Areas

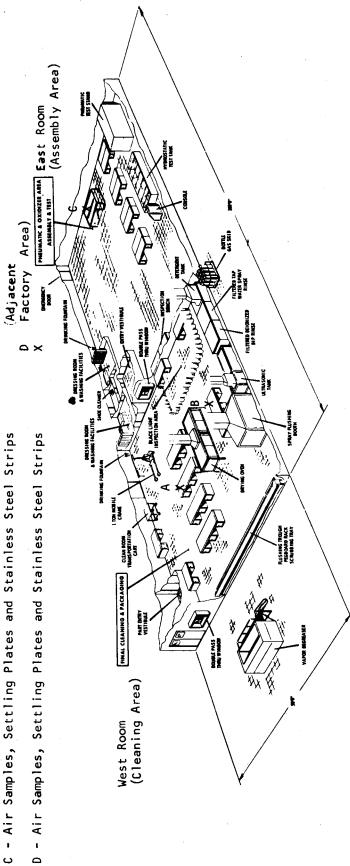
		nisms/Squa Aerobes	re Foot
	Bacteria	<u>Molds</u>	<u>Total</u>
Factory Area	14,000	5,000	19,000
Cleaning Area	440	160	600

Note: Each entry is an average of six determinations.

^{*} Exposure period from 47th to 52nd week of this study.

A + Air Samples and Settling Plates

B - Stainless Steel Strips



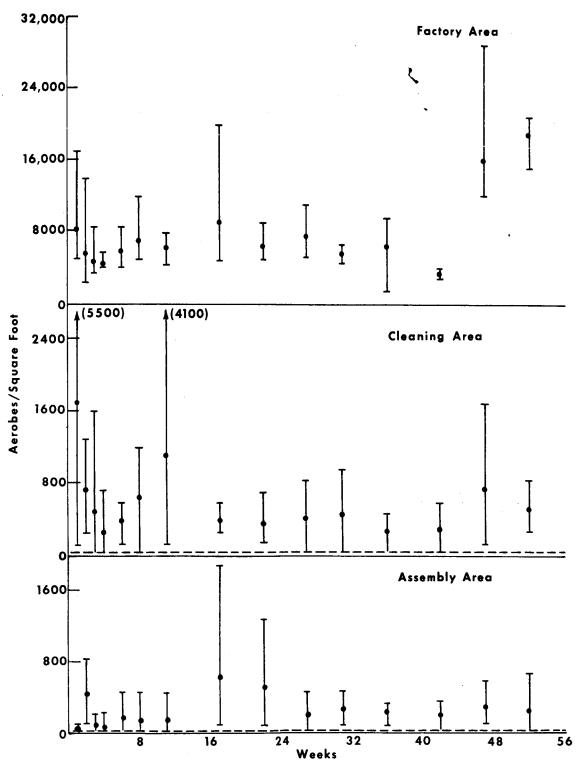


Figure 2. Number of Aerobes on Stainless Steel as a Function of Time

(Each bar shows the range of five determinations; the mean
of each is represented by a dot on the bar. The dotted line
indicates the lowest limit of contamination detected by the
assay method.)